

Viral Pathogens and Microbiological Indicators in Ground Water from Small Public Water Supplies

By **Donna S. Francy**¹, Rebecca N. Bushon¹, Emma J. Luzano¹, Erin Bertke¹, and G. Shay Fout²

¹U.S. Geological Survey, Columbus, OH

²U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Cincinnati, OH

Although this work was reviewed by EPA, it may not necessarily reflect official Agency policy.

ABSTRACT

Thirty-eight public ground-water-supply wells serving less than 3,300 people were sampled from July 1999 through July 2001 in southeastern Michigan to determine (1) occurrence of viral pathogens and microbiological indicators, (2) whether indicators are adequate predictors of the presence of viruses, and (3) factors that affect the presence of viruses. Samples were analyzed for enteric viruses by reverse transcriptase-polymerase chain reaction (RT-PCR), for culturable viruses by cell culture, and for the indicators total coliforms, *E. coli*, enterococci, and F-specific and somatic coliphage. Ancillary environmental and water-quality data also were collected.

A total of 169 regular samples and 32 replicate samples were collected. Each well was sampled from one to five times. By use of RT-PCR, enterovirus was found in four wells (10.5%) and hepatitis A virus (HAV) in five wells (13.2%). Culturable viruses were found once in two wells (5.9%), and neither of these wells was positive for viruses by use of RT-PCR. Nine of the 38 wells (23.7%) were positive for viruses by either RT-PCR or cell culture. One or more indicators were found in 18 of 38 wells. Total coliforms, *E. coli*, enterococci, and F-specific and somatic coliphage were found in 34.2, 10.5, 15.8, 5.9, and 5.9%, respectively, of the wells tested. Five out of nine (55.6%) virus-positive wells were also found to be positive for an indicator.

More virus-positive samples were found at sites served by septic systems than those served by sewerlines. Statistically significant relations were found between total coliforms and dissolved organic carbon, iron, or chloride concentrations. Presence of nitrate was related to presence of *E. coli*, enterococci, coliphage, or enteric viruses but not to total coliforms.

This study provides evidence for fecal contamination of ground water at small public-supply wells and shows the importance of collecting multiple samples at each site. The study also suggests that the collection of site-characteristic data, data on multiple water-quality parameters, and data on microbiological indicators is important for making better predictions of the presence of enteric viruses in small public water systems.

INTRODUCTION

- Insufficient monitoring information is available on the occurrence of pathogenic viruses in ground water and the factors that affect the vulnerability of ground water to fecal contamination.
- Those served by small public ground-water supplies (serving fewer than 3,300 people), may be at higher risk for microbiological contamination than for larger systems:
 - o Monitoring is less frequent than larger supplies
 - o Most small supplies are not disinfected

OBJECTIVES

Determine in small public ground-water supplies:

- 1. Occurrence of viral pathogens and microbiological indicators
- 2. Whether bacterial and viral indicators are adequate predictors of the presence of viruses
- 3. Environmental and water-quality factors that affect the presence of viruses

METHODS

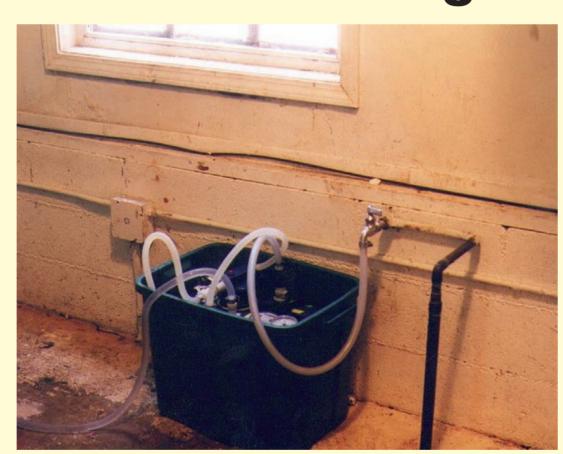
- Wells were located in the outskirts of a rapidly developing metropolitan area.
- Thirty-eight drinking-water wells were sampled from 1 to 5 times each; most wells were sampled 5 times.
- Wells produce water from discontinuous sand and gravel glacial aquifers screened from 50 to 150 ft deep. Wells were unconfined, semiconfined, or confined by poorly permeable glacial till.

Wells

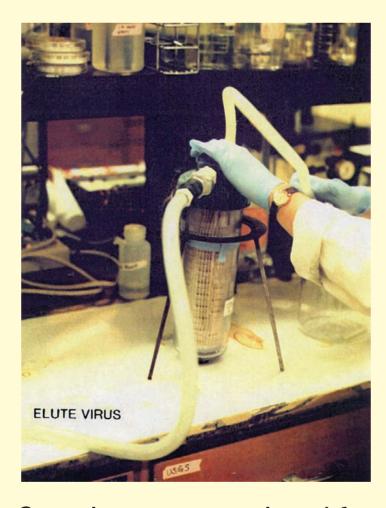


Wells were community or non-community water systems and included subdivisions, campgrounds, gas stations, factories, schools, and retirement homes.

Pathogenic viruses

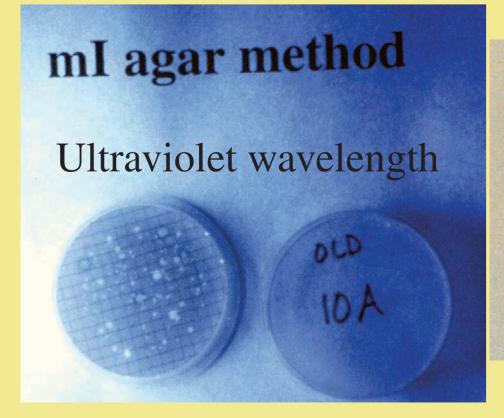


A volume 2,000 liters of water was concentrated onto a filter using a specially designed sampling device.

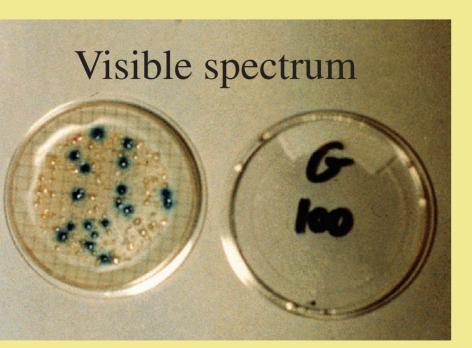


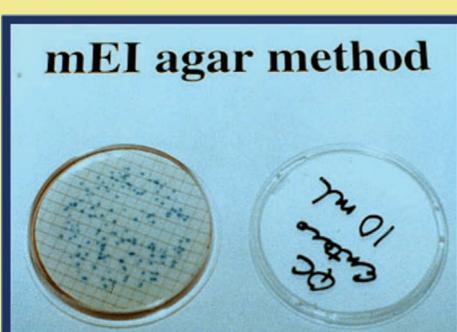
Samples were analyzed for pathogenic viruses by use of RT-PCR and cell culture.

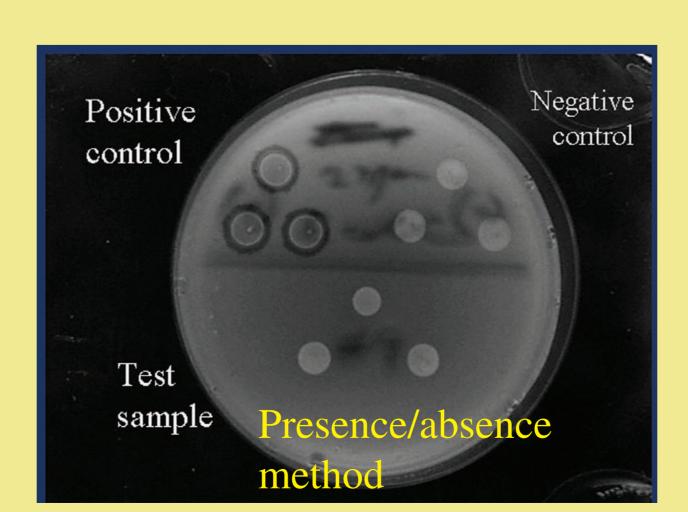
Microbiological indicators



One liter of water was collected for bacterial indicators total coliforms, *E. coli*, and enterococci.







Two liters of water were collected for somatic and F-specific coliphage.

RESULTS

RT-PCR (by sample)

Virus	Positive	Negative	Unknown	Percent unknown
Enterovirus	4	154	11	7%
Hepatits A virus	5	176	20	10%
Reovirus	0	69	19	22%
Rotavirus	0	54	34	39%
Norwalk virus	0	40	48	55%

Enterovirus and hepatitis A virus were found in less than 3% of samples. Unknown results were caused by failed quality-control tests and were highest for Norwalk virus.

Microbiological Indicators and Culturable Viruses

	Well Results		Sample Results			
Indicator or pathogen	Number of Wells Analyzed	Positive Results	% Positive	Numer of Samples Analyzed	Positive Results	% Positive
Total Coliforms	38	13	34.2%	167	14	8.4%
E. coli	38	4	10.5%	167	4	2.4%
Enterococci	38	6	15.8%	167	7	4.2%
F-specific coliphage	34	2	5.9%	121	2	1.7%
Somatic coliphage	34	1	2.9%	121	1	0.8%
Viruses by cell culture	34	2	5.9%	93	2	2.2%

Total coliforms were found most often among microbiological indicators. Culturable viruses were found in only 5.9% of the wells or 2.2% of the samples.

- Only 2 of 11 virus-positive samples were also positive for an indicator.
 - o 1 had culturable viruses and enterococci
 - o 1 had enterovirus by RT-PCR and total coliforms
- Five of nine virus-positive wells were also positive for an indicator, but not necessarily on the same sampling date:
 - o 2 had total coliforms
 - o 1 had E. coli
 - o 1 had enterococci
 - o 1 had F-specific coliphage

Factors Affecting the Presence of Viruses

Virus detection	Sewerage-system type				
	Septic system	Sewerline			
Not detected	13	16			
Detected	7	2			
p = 0.088					

A greater number of virus detections were found at sites with septic systems than with sewerlines.

CONCLUSIONS

- Viruses were found in 9 of 38 wells sampled (23.7%).
- Repeat sampling at the well is needed because of the intermittent occurrence of indicators and viruses.
- Indicators were fair predictors of the presence of viruses in wells (5 out of 9), but not in samples (2 out of 11).
- Some factors were related to the presence of indicators or viruses—sewerage system type, nitrate plus nitrite, chloride, dissolved organic carbon, iron.
- Some factors were not related to the presence of indicators or viruses—casing depth, percent clay, well age, land use.

The collection of site-characteristic data, data on multiple water-quality parameters, and data on multiple microbiological indicators is important for making better predictions of the presence of enteric viruses in small public water systems.